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Membrane dipole modifiers modulate single-length nystatin channels via reducing elastic stress in the vicinity of the lipid mouth of a pore

Evgeny G. Chulkov *, Ludmila V. Schagina, Olga S. Ostroumova

Institute of Cytology of the Russian Academy of Sciences, Tikhoretsky Ave. 4, St. Petersburg 194064, Russia

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ABSTRACT

The polyene antifungal antibiotic nystatin confers its biological activity by forming pores in the membranes of target cells. Exposure of only one side of the membrane to nystatin is more relevant than two-side exposure because in vivo antibiotic molecules initially interact with cell membrane from the exterior side.

The effect of flavonoids and styryl dyes on the steady-state conductance induced by a *cis*-side addition of nystatin was investigated by using electrophysiological measurements on artificial membranes. The assessment of changes in membrane dipole potential by dipole modifiers was carried out by their influence on K^+-nonactin (K^+-valinomycin) current. The alterations of the phase segregation scenario induced by nystatin and flavonoids were observed via confocal fluorescence microscopy.

The introduction of phloretin, phlorizin, biochanin A, myricetin, quercetin, taxifolin, genistin, genistein, and RH 421 leads to a significant increase in the nystatin-induced steady-state transmembrane current through membranes composed of a mixture of DOPC, cholesterol and sphingomyelin (57:33:10 mol%). Conversely, daidzein, catechin, trihydroxyacetophenone, and RH 237 do not affect the transmembrane current. Three possible mechanisms that explain the observed results are discussed: changes in the membrane dipole potential, alterations of the phase separation within the lipid bilayer, and influences of the dipole modifiers on the formation of the lipid mouth of the polyene pore.

Most likely, changes in the monolayer curvature in the vicinity of *trans*-mouth of a nystatin single-length channel prevail over alterations of dipole potential of membrane and the phase segregation scenarios induced by dipole modifiers.

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1. Introduction

Nystatin is one of the most commonly used antibiotics to treat human fungal infections [1–3]. Polyene antibiotics predominantly impart their biological activity via pore formation in the membranes of target pathogenic cells [2,4]. One-side addition of polyenes to the membrane is considered to be biologically relevant because in vivo nystatin molecules initially interact with the cell membrane from the exterior surface of the membrane. Kleinberg and Finkelstein [5] hypothesized that nystatin forms two distinct types of channels in sterol-containing lipid membranes. That is, when nystatin is added to one side of planar lipid membranes, single-length channels form; when added to both sides, double-length channels form. The steady-state transmembrane current induced by one-side addition of nystatin is achieved in tens of minutes or faster; in contrast, addition to both sides requires hours to reach a steady-state [6]. In addition, different concentrations of nystatin in membrane bathing solutions are required to induce measurable transmembrane currents. The sidedness of nystatin addition leads to the formation of channels with different selectivities, which suggests that they are significantly different species [2,5,7].

Despite the high therapeutic efficiency of polyenes, serious side effects limit their pharmaceutical applications [3,8,9]. One possible method to improve their therapeutic efficiency is to use a combination of polyenes and other biologically active agents that may enhance the activity of polyenes. To this end, membrane dipole modifiers, such as flavonoids and styryl dyes, are especially attractive because their influence on the channel forming activity of several different antimicrobial agents has been established [10–18]. Flavonoids are a class of polyphenols that are found ubiquitously in plants. Their biological activity is related to antioxidant, anti-allergic, anti-inflammatory, antimicrobial, and anticancer properties [19–21]. In vitro studies indicate that some flavonoids alter lipid packing [22–24] and decrease the membrane dipole potential [25,26]. Synthetic styryl dyes are widely used in bio-labeling and in medicinal analysis [27] and may be applied for the modulation of membrane properties [17,18]. As shown by Ostroumova et al. [18] flavonoids and some other dipole modifiers may alter the steady-state transmembrane current induced by the two-sided addition of polyenes.

* Corresponding author. Tel.: +7 812 2972460; fax: +7 812 2970341.
E-mail address: evchulkov@mail.com (E.G. Chulkov).

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In this paper, some possible mechanisms of action that explain the effects of the dipole modifiers on the transmembrane conductance induced by one-sided action of nystatin are considered. First, changes in the dipole potential may potentiate channel formation via electrostatic interactions. Second, the influences of amphiphilic compounds on the lateral phase separation of lipids within the membrane may expand the area containing the phase that polyene antibiotic prefers. Third, interactions of the dipole modifiers with the channel may lead to the stabilization of the lipid mouth of a pore.

2. Materials and methods

All of the chemicals were reagent grade. 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol (Chol), ergosterol (Erg), brain bovine sphingomyelin (SM), and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rh-DPPE) were obtained from Avanti Polar Lipids, Inc. (Pelham, AL). Dimethylsulfoxide (DMSO), phloretin (3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)-1-propanone), phlorizin (1-[2-(β-D-glucopyranosyloxy)-4,6-dihydroxyphenyl]-3-(4-hydroxyphenyl)-1-propanone), biochanin A (5,7-dihydroxy-4′-methoxyisoflavone), myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone), quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one), taxifolin ((2R,3R)-3,3′,4′,5,7-pentahydroxyflavanone, (2R,3R)-dihydroquercetin), genistin (genistein-7-O-β-D-glucopyranoside), genistein (5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), daidzein (4′,7-dihydroxyisoflavone, 7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, 7-hydroxy-3-(4-hydroxyphenyl)chromone), catechin ((2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-1(2H)-benzopyran-3,5,7-triol), 2′,4′,6′-trihydroxy-acetophenone monohydrate (THAP), triton X-100 (TX-100), and nystatin A were purchased from Sigma Chemical (St. Louis, MO). RH 237 (N-(4-sulfobutyl)-4-(6-(4-(dibutylamino)phenyl)hexatrienyl)pyridinium, inner salt) and RH 421 (N-(4-sulfobutyl)-4-(4-(4-(dipentylamino)phenyl)butadienyl) pyridinium, inner salt) were purchased from Molecular Probes (Eugene, OR). The water used in this study was double distilled and deionized. The KCl solutions were buffered with 5 mM HEPES, pH 7.0. The chemical structures of the flavonoids, RH dyes, and nystatin are shown in Fig. 1.

Planar lipid bilayers were formed using a monolayer-opposition technique [28] on a 50-μm-diameter aperture in a 10-μm-thick Teflon film that separated the two (*cis*- and *trans*-) compartments of the Teflon chamber. The volume of chamber each was 1.5 ml. The aperture was pretreated with hexadecane. Lipid bilayers were made from 57 mol% DOPC or DOPE, 33 mol% sterol (Chol or Erg), and 10 mol% SM. After the membrane was completely formed, nystatin, from a stock solution (20 mM in DMSO), was added to the *cis*-compartment to a final concentration that ranged from 20 to 40 μM. Ag/AgCl electrodes with agarose/2 M KCl bridges were used to apply the transmembrane voltage (V) and

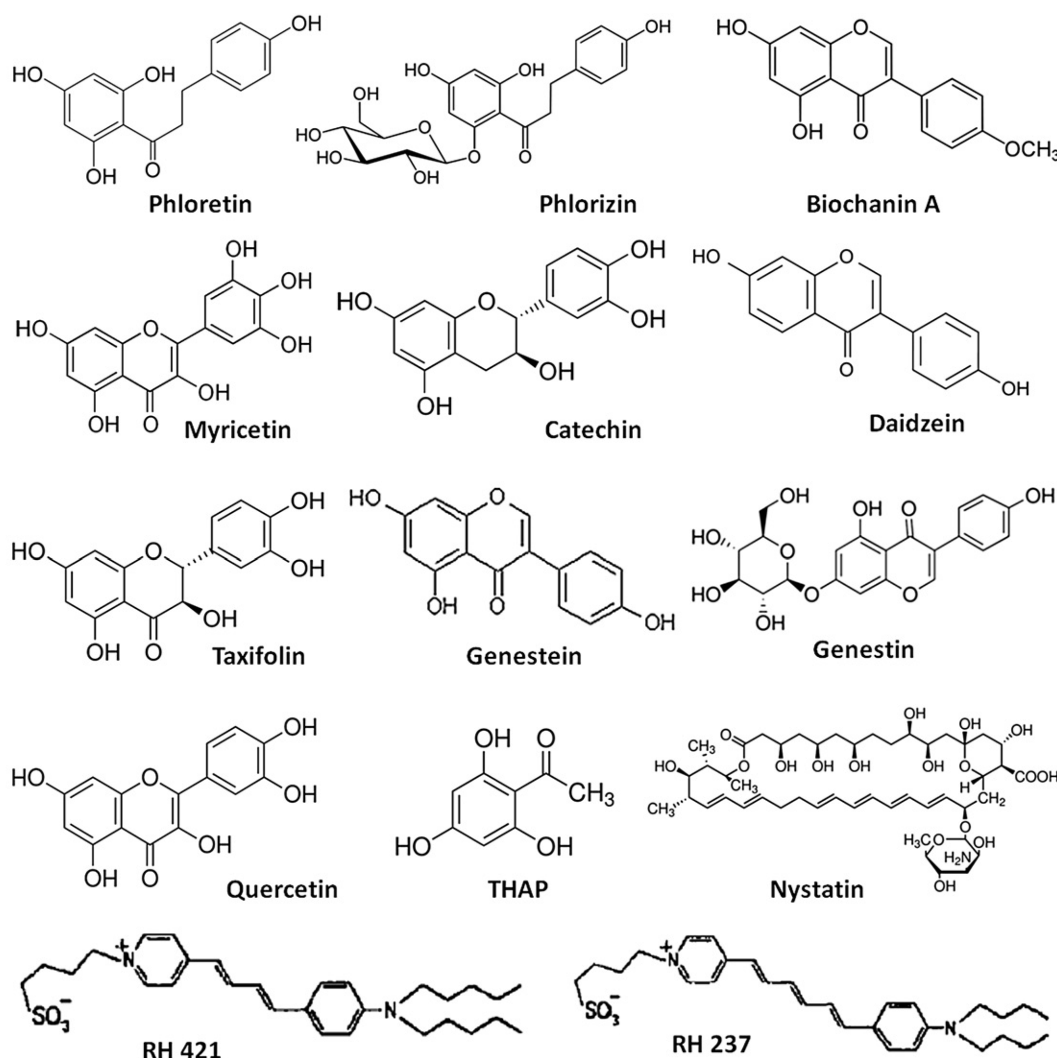

Fig. 1. Chemical structures of tested dipole modifiers and nystatin.

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